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NATIONAL STANDARD METHOD

ISOLATION OF ENTEROVIRUSES AND PARECHOVIRUSES

VSOP 24

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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

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Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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The HPA aims to be a fully Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions[1].

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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AMENDMENT PROCEDURE

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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
3/ 09.11.09	2	3	11	**References**	References reviewed and updated

ISOLATION OF ENTEROVIRUSES AND PARECHOVIRUSES

SCOPE OF DOCUMENT

This National Standard Method describes the isolation and identification of enteroviruses and parechoviruses from clinical material.

INTRODUCTION

Background

In the genera *Enterovirus* and *Parechovirus* in the family *Picornaviridae* there are over 60 serotypes that can infect humans. The human enteroviruses are classified into 5 species, the polioviruses and the human enteroviruse groups A-D[2]. Before the more recent molecular classification subgroups were based on serotype relationships and included polioviruses (3 serotypes), Coxsackie A and B (23 and 6 serotypes respectively), echoviruses (26 serotypes) and the newer numbered enteroviruses types 68-71. Two viruses formerly classified with the echoviruses, echovirus 22 and 23, were shown to comprise a separate genus within the *Picornaviridae* family, the genus *Parechovirus*; these viruses are now referred to as human parechovirus types 1 and 2. Discovery and characterisation of picornaviruses has progresssed rapidly with the introduction of molecular testing and molecular typing schemes and there are now over 90 viruses classed as enteroviruses and 6 parechovirus types have been described. Throughout this document the term 'enterovirus' will be used generically to cover both enteroviruses and parechoviruses.

The enteroviruses can cause a wide spectrum of human illness, from mild non-specific fever and rash to upper respiratory tract infections, aseptic meningitis, and pleurodynia, through to life-threatening infections such as myocarditis, encephalitis and paralytic poliomyelitis. The majority of infections however are asymptomatic.

Diagnosis of enterovirus infection by isolation in cell culture is of diminishing importance, it was relatively quick and the isolate could be typed using antisera for epidemiological purposes. However PCR methods are being used increasingly. These are generally more sensitive and more rapid than cell culture methods and can detect infections with uncultivable enteroviruses[3,4].

1 SAFETY CONSIDERATIONS[5-13]

1.1 SPECIMEN COLLECTION

Appropriate hazard labelling according to local policy should be used.

1.2 SPECIMEN TRANSPORT AND STORAGE

Compliance with current postal and transport regulations is essential.

A suitable virus transport system must be used (where appropriate) and the specimen placed in a sealed plastic bag or pouch.

Appropriate hazard labelling according to local policy should be used.

1.3 SPECIMEN PROCESSING

- All enteroviruses are in Hazard Group 2. Laboratory procedures that may give rise to infectious aerosols must be conducted in a microbiological safety cabinet
- Vaccination against poliovirus is required; guidance is given in the Health Protection Agency immunisation policy
- Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this National Standard Method
- Please note that laboratories retaining wild poliovirus infectious or potentially infectious materials are recommended to operate under biosafety level 2/polio (BSL-2/polio)[14]

The above guidance should be supplemented with local COSHH and risk assessments

2 SPECIMEN COLLECTION

2.1 TYPE OF SPECIMENS

Throat swab	Vesicle swab
Eye swab	CSF
Faeces	Pericardial fluid
Rectal swab	Biopsy tissue
Vesicle fluid	Post mortem tissue

2.2 OPTIMAL TIMING OF SPECIMEN COLLECTION

Swabs, scrapings, pericardial fluid and tissue should be taken as soon as possible after symptoms appear and put immediately into Virus Transport Medium (VTM). Faeces and CSF should be collected in CE marked leak proof containers[a] sealed in plastic bags.

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be processed as soon as possible. Ideally, transportation systems should ensure that specimens arrive in the laboratory within 24 hours.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Specimens should be placed in a suitable VTM immediately after collection.

When there is a delay in processing, specimens should be refrigerated. If the delay is likely to exceed 24 hours, samples should be frozen at -70°C, or lower, and thawed immediately prior to processing. Repeated freezing and thawing should be avoided.

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

In the past enterovirus isolation in cell culture was the most widely used method for diagnosis. PCR techniques now offer significant advantages in terms of sensitivity and turnaround time. Although PCR does not provide an isolate for typing specialist centres may still be able to infer the species type by gene sequencing of the original sample[16].

4.2 CULTURE AND INVESTIGATION

4.2.1 SAMPLE PREPARATION

Faeces

Faecal specimens are made into a 10-20% suspension in a balanced salts solution with antibiotics, then clarified by centrifugation at 1600-2000 g for 10 minutes.

Tissues

Tissues are ground in a sterile mortar. Occasionally the addition of sterile sand and a small amount of medium is required.

After making a 10-20% suspension in VTM, clarification by centrifugation should occur at 1600-2000 g for 10 minutes.

Swabs

Swabs should be agitated to release cellular material into the virus transport medium, taking care not to produce aerosols.

CSF and Pericardial fluid specimens

These are considered pure samples and require only minimium preparation.

4.2.2 ISOLATION

Many of the cell lines in routine use for virus isolation are susceptible to infection with most enteroviruses. Of the commonly used cell lines, primary rhesus monkey kidney (RMK) are the most susceptible but have not been available since April 2006 for ethical reasons. The human diploid cell lines eg MRC-5 have been found to be equally susceptible and to show cytopathic changes more rapidly than other cell lines[17]. RD (rhabdomyosarcoma) cells have also been found to be susceptible to many enteroviruses (exception being the Coxsackie B group) including a number of the Coxsackie A group which normally are only isolated in suckling mice[18]. Multiple cell lines should be used in order to increase the yield and enhance the rapidity of enterovirus isolation.

Inoculate up to 0.2 mL CSF, faecal extract or VTM containing the clinical material into each of the two cell lines. The cells should be incubated at 35 – 37°C, for up to ten days. They should be examined at regular intervals for the appearance of cytopathic changes characteristic of enteroviruses.

4.3 IDENTIFICATION

4.3.1 WITHIN THE LABORATORY

The growth of enteroviruses in cell culture may be identified through the appearance of characteristic cytopathic changes and confirmed by immunofluorescence or neutralisation.

Serotyping of enteroviruses should be performed, principally to exclude poliovirus. This is commonly done using indirect immunofluorescence with type-specific monoclonal antibodies to determine the enterovirus group, echovirus, coxsackie B virus and poliovirus. Poliovirus should be further typed to determine serotype 1-3.

Further typing may be carried out if required by indirect immunofluorescence or neutralisation tests using the Lin, Benyesh, Melnick (LBM) combination neutralising serum pools or other commercial reagents[19].

Preparation and staining of samples for typing by immunofluorescence using commercial reagents should be carried out strictly in accordance with the manufacturer's instructions.

4.4 REFERRAL TO REFERENCE LABORATORIES

All polioviruses and unidentified viruses for typing must be referred to CfI. It is important that surveillance continues to find and to differentiate vaccine and wild type poliovirus isolates. For further information on polioviruses refer to QSOP 31 – Survelliance of polio in the UK.

5 QUALITY ASSURANCE

A quality system should be in place to ensure that appropriate internal and external quality assessment and quality control procedures are maintained. For further information on quality assurance refer to QSOP 27 – Quality assurance in the diagnostic virology and serology laboratory.

It is essential that laboratories have evidence of adequate validation of methods, equipment and commercial and in-house test procedures demonstrating that they are fit for purpose[20].

6 LIMITATIONS

Successful isolation of organisms depends on correct specimen collection, transport, storage and processing; the quality and range of cell lines used and the use of correct conditions for culture and the provision of adequate/suitable clinical information.

Only cell lines proven to be susceptible to infection with enterovirus should be used. Susceptibility should be checked on acquisition and at regular intervals while in use. Cells removed from liquid nitrogen storage should be checked for sensitivity before use.

The procedure(s) in these documents aim to describe good microbiological standard methods for the specimen types specified. Other procedures may be required and professional interpretation by qualified staff is essential. Please note that knowledge of infectious diseases changes constantly and although this National Standard Method is regularly reviewed it may not include emerging pathogens.

7 REPORTING PROCEDURE

Negative specimens should be reported as "Virus not isolated"

Positive specimens should be reported as "Enterovirus (type xx) isolated" or "Enterovirus isolated. Typing results to follow"

8 REPORTING TO THE HPA[21]

Any positive results should be reported to CfI in accordance with published guidelines.

9 RELEVANT NATIONAL STANDARD METHODS

For additional details on specific areas of diagnosis refer to the relevant NSMs available through the Department for Evaluations, Standards and Training web page (www.hpa-standardmethods.org.uk).

Other documents that may be of relevance to this NSM are:

QSOP 27 – Quality assurance in the diagnostic virology and serology laboratory

QSOP 31 – Survelliance of polio in the UK

10 ACKNOWLEDGEMENTS AND CONTACTS

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[a] *The requirements of the EU in vitro Diagnostic Medical Devices Directive[15] (98/79/EC Annex 1 B 2.1) state that such devices must "reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".*